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Failed endometrial metabolite synthesis contributes to subfertility in women with unexplained infertility

Endometrium spectroscopy

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Abstract

Aim: This study compares endometrial metabolite signal intensities of patients diagnosed with unexplained infertility (UEI) with those of fertile women.

Material and Methods: A total of 20 patients diagnosed with UEI and 20 fertile women with at least two children were included in the study. UEI patients were matched with the fertile group in terms of age and BMI. UEI was defined as the absence of conception despite 12 months of unprotected intercourse, not explained by anovulation. Endometrial spectroscopy was applied to the women in both groups in the mid-luteal phase of the cycle. The endometrial signals of choline (Cho), creatine (Cr), lactate, and lipids were measured in units and denominated in parts per million.

Results: All patients in both groups underwent successful spectroscopy and the main metabolites of Cho, Cr, lactate, and lipids were recorded. Cho (2.93±1.03 vs. 3.22±2.04, $p<0.05$) and Cr (2.33±1.30 vs. 2.76±1.43, $p<0.01$) metabolite signal intensities were found to be significantly lower in UEI patients compared to fertile controls. Lactate (0.56±0.11 vs. 0.62±0.30, $p>0.66$) and lipid (0.70±0.02 vs. 0.78±0.61, $p>0.54$) signal intensities were found to be similar between the groups.

Discussion: Decreased Cho and Cr signals, which are physiological indicators of endometrial function, may be the possible cause of subfertility in UEI patients.

Keywords

Unexplained Infertility, Choline, Creatine, Endometrium, Spectroscopy

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Introduction

About 10-30% of couples with normal fallopian tubes, ovulatory functions, and semen analysis results are diagnosed with unexplained infertility (UEI) [1, 2]. The mechanisms that cause infertility in this patient group are not clearly known. Some physicians use standby therapy, while others offer intrauterine insemination or IVF. The application of different treatments is due to the lack of useful data on the underlying cause of UEI. The only pleasing aspect of the diagnosis of UEI is that these patients have a 13-27% chance of spontaneous pregnancy [3]. Endocrinological disorders, immunological defects, and genetic causes have been considered among the potential causes of UEI [4]. The least studied area in the etiology of UEI is the endometrium. The reason for this is mostly due to the fact that the endometrium is evaluated only as thin or thick in ultrasonographic evaluations, and further evaluations are done late due to the invasiveness of procedures such as hysteroscopy and endometrial biopsy. Although a thin endometrium is weak in terms of receptivity, the presence of a thick endometrium does not always predict successful implantation. It is obvious that there is a need for a test to evaluate endometrial functions noninvasively in cases of UEI. In this context, the only method by which we can evaluate the endometrium noninvasively is spectroscopy [5].

About a decade ago, it was reported that MR spectroscopy is an effective method for evaluating the physiological cycle of the endometrium [5-7]. The feasibility of spectroscopy in the evaluation of the endometrium was confirmed by subsequent studies [8]. Choline (Cho), creatine (Cr), lactate, and lipid signals to be obtained by spectroscopy will provide us with clues about the physiopathological changes that occur at the cellular level in the endometrium [5-7]. However, it remains to be determined whether spectroscopy of the endometrium can help the physician in having knowledge about the status of endometrial function in cases of UEI. If the data to be obtained with this noninvasive method are meaningful, it may provide a new perspective in the diagnosis and treatment of UEI. This case-controlled study was planned to compare the endometrial metabolite signal types of UEI patients with the signal characteristics of fertile women.

Material and Methods

A total of 20 women diagnosed with UEI and 20 fertile women with at least two children were included in the study. UEI patients were matched with the fertile group in terms of age and BMI. UEI was defined as the absence of conception despite 12 months of unprotected intercourse, not explained by anovulation. In addition to ovulatory values of serum luteinizing hormone (LH) surge or mid-luteal progesterone presence and normal semen parameters according to World Health Organization criteria, the presence of patent fallopian tubes during hysterosalpingogram or laparoscopy was considered in the diagnosis of UEI. Patients with endometrial polyps, leiomyoma, adhesion, and congenital uterine anomaly were excluded from the study. Patients diagnosed with hydrosalpinx, endometrioma, or endometriosis were also excluded, as were anovulatory PCOS patients. Patients in both groups underwent endometrial MR spectroscopy in the mid-luteal phase of the cycle. Spectroscopy was performed 7-9 days after the LH surge. LH surge was considered as a twofold or more increase in serum LH levels compared to the average of the previous three days. Spectroscopy analysis of the endometrium was performed using MR imaging. T1-weighted and T2-WI images with 4-mm-thick sections were analyzed in the axial and coronal planes. Multi-voxel point-resolved spectroscopy techniques with short and long TEs were applied. The metabolite peaks of endometrial cells were analyzed with Magnetic Resonance User Interface software. The endometrial signals of Cho, Cr, lactate, and lipids were measured in units and denominated in parts per million. First, the endometrium was visualized in both the UEI and fertile control groups by magnetic resonance imaging before the voxel was inserted. The volume of interest of the voxel was placed at the center of the endometrium. Care was taken to prevent abdominal or myometrial tissue from entering the voxel area. Endometrial metabolites were quantitatively analyzed from spectroscopic images and recorded as ppm. Metabolite peaks included Cho located at 3.2 ppm, Cr located at 3-3.1 ppm, lactate located at 1.4 ppm, and a compound peak containing lipids and lactate located at 0.8-1.4 ppm. The signal intensities of Cho, Cr, lactate, and lipids in UEI were analyzed and compared with the results of fertile women. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and approval for the study was obtained from the institutional review board.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Normally distributed variables were analyzed with the independent samples t-test. Non-normally distributed variables were analyzed with the Mann-Whitney U test. Categorical variables were analyzed with chi-square tests. The correlations between demographic variables, hormonal parameters, and metabolite values were evaluated using Pearson correlation coefficients. Data are given as mean ± standard deviation for continuous variables according to normality of distribution and as frequency for categorical variables. Values of $p<0.05$ were accepted as statistically significant.

Results

Participants in both groups were similar in terms of age (27.4±1.04 vs. 28.1±0.2 years, $p>0.61$) and BMI (27.1±1.03 vs. 26.5±1.91, $p>0.40$). There was no significant difference between the two groups in terms of other demographic and hormonal parameters. While 13 of the women in the fertile group had delivered by cesarean section, 7 had a history of normal vaginal delivery. All of the women in both groups underwent successful spectroscopy and the levels of the main metabolites of Cho, Cr, lactate, and lipids were obtained. Cho (2.93±1.03 vs. 3.22±2.04, $p<0.05$) and Cr (2.33±1.30 vs. 2.76±1.43, $p<0.01$) metabolite signal intensities were found to be significantly lower in UEI patients compared to fertile controls. Lactate (0.56±0.11 vs. 0.62±0.30, $p>0.66$) and lipid (0.70±0.02 vs. 0.78±0.61, $p>0.54$) signal intensities were found to be similar between the groups. The most prominent metabolite peaks in the fertile group were Cho and Cr, respectively. In UEI patients, these two metabolite peaks were either weak in intensity or the signals in

spectroscopy did not show clear peaks. Lactate and lipid peaks, which are indicators of endometrial hypoxia and necrosis, were found to be very weak in both fertile women and UEI patients. There was no difference between the groups in terms of lactate and lipid metabolites, which are endometrial pathology peaks. There was no significant correlation between demographic and laboratory parameters and metabolite signals of the women in either group.

Discussion

Approximately 30-40% of patients followed with a diagnosis of infertility are examined under the title of "unexplained infertility," since no underlying pathology was detected as a result of the initial infertility tests [4, 9]. Most clinicians believe that women in this patient group can conceive spontaneously or with different treatments. Indeed, about one-third of these patients may become pregnant within a certain period of time. However, the remaining two-thirds have to pursue assisted reproductive techniques because they have difficulty in conceiving. What could be the mechanism that prevents pregnancy in these women with normal semen analysis, normal tubal and uterine cavity passages, and healthy ovulation? The first answer that comes to mind is the endometrium, because the endometrium is the only region that is not taken into consideration when diagnosing UEI. The absence of space-occupying lesions such as polyps and myomas in the cavity and the detection of a triple-line endometrium of sufficient thickness on ultrasonography does not mean that everything is suitable for implantation. In many diseases such as PCOS and endometrioma, which cause infertility, although the endometrium appears morphologically healthy, it is found to have some defects at molecular and/or genetic levels in histopathological and RT-PCR examinations [10]. Since the ovulatory function and tubal passage are normal in UEI cases, we should expect the embryo to attach to the endometrium. In order for the endometrium to accept the embryo, it must have undergone adequate decidualization, developed pinopodes, and have upregulated sex steroid receptors. For all these changes to occur, sufficient energy production must be ensured at the cellular level in the endometrium, membrane integrity must be preserved, and necrotic events with hypoxia and tissue death must not occur [5-7].

Cho is the main indicator of cell membrane integrity and regeneration capacity. Cr is another metabolite that shows that the energy pathways necessary for the realization of vital functions in the cell are active [5,7,11]. In cells with a healthy life cycle, both Cho and Cr signals should be of sufficient intensity. In contrast to the patients in the fertile control group, we found that both Cho and Cr signals were significantly reduced in UEI patients. Decreased Cho and Cr metabolite levels in tissue with a dense mitotic capacity, such as the endometrium, indicate a problem at the cellular level. Although the endometrium appears to be morphologically normal in cases of UEI, decreased Cho and Cr signals suggest that there is a problem in both endometrial mitosis and the decidualization process [12, 13]. Since the endometrium is associated with the brain via follicle-stimulating hormone (FSH), LH, and the ovaries via estrogen and progesterone, the decrease in Cho and Cr signal intensities may be of central or ovarian origin. Subtle changes in FSH and LH secretion or defects in sex steroid synthesis and release that are not reflected in the laboratory may be the cause of decreased Cho and Cr signals. On the other hand, endometrial estrogen or progesterone receptor defects may also decrease the signal intensities of these two metabolites.

Since the lactate and lipid signal peaks of the fertile group and UEI patients were similar, there was no irreversible damage to the endometrium in these cases of UEI. Lactate is an indicator of anaerobic glycolysis. In the UEI group, lactate levels similar to those of the fertile group indicate the presence of sufficient oxygen in the endometrium for energy production [5, 11]. Lipid signals are indicative of the disruption of cell membrane integrity. The presence of the lipid signal at a level similar to the fertile group in UEI patients suggests that the membranes of the endometrial cells are healthy. All these findings suggest that UEI patients have a temporary impairment in energy production in the endometrium. The fact that many patients become pregnant spontaneously also supports the assumption that the problem in the endometrial cells is transient. If the diagnostic value of endometrial spectroscopy is clearly proven as a result of more comprehensive studies to be carried out by increasing the number of cases considered, infertility physicians will have a cheap, easily applicable, and non-invasive endometrial screening test for women with UEI.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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